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(54) PROCESS FOR PRODUCING MODIFIED KERATIN PROTEIN

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SPECIFICATION

1. Title Of The Invention

PROCESS FOR PRODUCING MODIFIED KERATIN PROTEIN

2. Claims

1. A process for producing modified keratin protein, in which a keratinous substance is subjected to reduction treatment using a reducing agent in water or a mixed solvent made up of water and a hydrophilic organic solvent to turn disulfide bonds into sulfhydryl groups and is subsequently reacted with a cationizing agent having quaternary nitrogen and groups represented by [*See first two structures on page 205 – trans.*] or CH_2=CH– in one molecule.
2. The production process as set forth in claim 1, in which the keratinous substance is wool, feather, hair, nail, or horn.
3. The production process as set forth in claim 1, in which the hydrophilic solvent is methanol, ethanol, *n*-propanol, isopropanol, acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, or hexamethylphosphoric triamide.
4. The production process as set forth in claim 1, in which the reducing agent is 2-mercaptoethanol, thioglycolic acid, tri-*n*-butylphosphine, triphenylphosphine, ascorbic acid, sodium hydrogensulfite, or sodium sulfite.

3. Detailed Explanation Of The Invention

The present invention relates to a process for producing modified keratin protein, and, more specifically, to a process for producing a modified keratin protein extremely soluble in water or a mixed solvent made up of water and a hydrophilic organic solvent, where the process is characterized in that, in order to recover protein from a keratinous substance, reduction treatment is carried out in water or in a mixed solvent made up of water and a hydrophilic organic solvent to cleave disulfide bonds (–S–S–) in keratin and produce sulfhydryl groups (–SH), and a quaternary ammonium salt

type cationizing agent is added to the resulting sulfhydryl groups, or said sulfhydryl groups and other functional groups in the protein, for example, hydroxyl, amino, carboxyl, and other functional groups.

One of the methods commonly used in the past for extracting protein from keratinous substances consisted in solubilizing the protein by cleaving the disulfide bonds in keratin by reduction or oxidation treatment. However, the use of copious amounts of various denaturants as solubilization adjuvants in the reduction treatment method makes the procedure extremely burdensome and complicated. For instance, in the reduction and solubilization treatment method based on the use of 2-mercaptoethanol, a reduction reaction is carried out in a strong aqueous solution of urea serving as a solubilization adjuvant, and after the reduction-treated keratin dissolves in the strong aqueous solution of urea, iodoacetic acid is added to sulfhydryl groups, which are unstable to oxidation, and a carboxymethylation reaction, etc. is carried out to produce a stable form of the protein. After that, the undissolved matter is partially removed by filtration, and low molecular impurities, mainly the considerable amount of urea present in the solution of protein in the aqueous urea solution, are removed from the aqueous solution using operations such as dialysis etc. Protein is recovered from the thus obtained aqueous solution of protein by isoelectric precipitation or lyophilization. On the other hand, there is a method, in which protein is recovered from keratinous substances by oxidation, which cleaves the disulfide bonds (–S–S–) to give sulfonic acid groups ($-SO_3H$). It is known, however, that oxidation using peracetic acid brings about hydrolysis of polypeptide bonds in the principal chain, which makes it undesirable as a method for recovering high molecular weight keratin protein.

In addition, the term "protein" used in the present invention refers not to low molecular weight oligopeptides with a typical molecular weight of several thousand or less obtained by hydrolyzing polypeptide bonds in the principal chain of the keratinous substance with acids, alkalis, or oxygen, etc., but mainly to protein with a molecular weight of at least 10,000 or more, preferably, with a molecular weight of 20,000 to 50,000, which is obtained by cleaving the disulfide bonds of the keratinous substance.

The thus obtained protein is supposed to be soluble, but because of the hydrogen bonds, ionic bonds, hydrophobic bonds, etc. in the protein, it is poorly soluble in water and organic polar solvents, and even when it is dissolved, only very weak solutions can be obtained. At the present time, this property of the protein presents serious obstacles to its possible use in various applications.

At present, there are two problems in the recovery and use of protein from keratinous substances, namely, the burdensome and complex nature of the recovery procedure and the poor solubility of the recovered protein.

The present inventors, as a result of in-depth investigations aimed at eliminating these two problems, discovered that a protein extremely soluble in water and mixed solvents made up of water and hydrophilic organic solvents is obtained by a simple procedure, by cleaving disulfide bonds in keratin by reduction and then reacting it with a quaternary ammonium salt type cationizing agent, and thus arrived at the present invention.

Namely, the present invention relates to a process for producing modified keratin protein extremely soluble in water and mixed solvents made up of water and hydrophilic organic solvents, in which, in order to recover protein from a keratinous substance, the keratinous substance is subjected to reduction treatment with a reducing agent in water or a mixed solvent made up of water and a hydrophilic organic solvent to turn disulfide bonds (–S–S–) in the keratinous substance into sulfhydryl groups (–SH) and a cationizing agent having quaternary nitrogen and groups represented by [*See first two structures on page 205 – trans.*] or CH_2=CH– in one molecule is subsequently

added to the resulting sulfhydryl groups, or said sulfhydryl groups and other functional groups in the protein, for example, hydroxyl, amino, carboxyl, and other functional groups.

Below, the present invention is explained in detail.

Wool, feather, hair, nail, horn, etc. can be suggested as the keratinous substances used as the raw material in the present invention, but wool and feather are the most common materials.

Any reducing agents capable of cleaving the disulfide bonds of keratinous substances into sulfhydryl groups are suggested as the reducing agents used in the present invention. 2-mercaptoethanol, thioglycolic acid and other alcohols, or thiol derivatives of carboxylic acids, tri-*n*-butylphosphine, triphenylphosphine, and other phosphorus compounds, ascorbic acid and other organic reducing agents, as well as sodium hydrogensulfite, sodium sulfite, and other inorganic reducing agents can be used as the above-mentioned reducing agents.

The reaction of reduction of the keratinous substance in the present invention can be carried out by publicly known prior-art methods, but it is preferable to avoid introducing solubilization adjuvants such as guanidine sulfate and urea into the reaction medium. This is due to the fact that such solubilization adjuvants must be removed when protein is recovered upon termination of the entire reaction, which is a major factor contributing to the complexity of the protein recovery procedure. In other words, in the present invention, only water or a mixture of water and hydrophilic organic solvents in any proportion should be used as the reaction medium. Methanol, ethanol, *n*-propanol, isopropanol, acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, hexamethylphosphoric triamide, etc. are suggested as examples of such hydrophilic organic solvents. Although there are no strict limitations on the amount of the reaction medium, the amount should be sufficient to conduct the reduction reaction in a uniform manner, the typically used amount being 10~100 times the amount of the keratinous substance by weight. The amount of the reducing agent used is generally in the range of from 2 to 10 times the equivalent required for the disulfide bonds in the keratinous substance used as the raw material. In addition, although there are no specific limitations on the pH of the reduction reaction system, it is necessary to select pH conditions, under which the keratinous substance does not substantially convert to oligopeptides, that is, typically in the range of from 2 to 12, and more preferably, 6 to 11.

Furthermore, as far as the reaction temperature is concerned, it is normally sufficient to conduct the reaction at room temperature. However, if necessary, it can be conducted under heating to shorten the time required for reduction. The reaction time has to be sufficient to bring the reaction to full completion and may vary depending on the reaction temperature, but usually the reaction requires 2 to 5 hours or more. In addition, it is preferable to conduct said reduction reaction in an atmosphere of an inert gas such as nitrogen. This is due to the fact that the sulfhydryl groups produced by the reduction reaction are very easily oxidized and are unstable to oxygen in the air.

Glycidyltrimethylammonium chloride, glycidyltriethylammonium chloride, 3-chloro-2-hydroxypropyltrimethylammonium chloride, allyltrimethylammonium chloride and corresponding bromides and iodides, etc. are suggested as the quaternary ammonium salt type cationizing agents used in the present invention, with glycidyltrimethylammonium chloride being the most commonly used agent.

The reaction of addition of the quaternary ammonium salt type cationizing agent to the reduction-treated keratinous substance used in the present invention is carried out by adding the quaternary ammonium salt type cationizing agent as is to the reduction reaction medium upon termination of the reduction reaction. The added quaternary ammonium salt type cationizing agent undergoes an addition reaction with the functional groups in the produced protein, primarily with the sulfhydryl groups. However, when added in excess of the equivalent required for the sulfhydryl

groups, it undergoes an addition reaction with functional groups other than the sulfhydryl groups, for example, such as hydroxyl, amino, carboxyl, and other groups. The appropriate amount of the added quaternary ammonium salt type cationizing agent is 0.1 to 6 times, and, more preferably, 0.5 to 2 times the equivalent required for the sulfhydryl groups produced by reduction in the keratinous substance. When it is less than 0.1, it is impossible to achieve sufficient solubility of the resulting modified keratin protein in water and mixed solvents made up of water and hydrophilic organic solvents, and when it is more than 6, the intrinsic properties of the protein are impaired, which is undesirable. In the range of from room temperature to 90°C, any temperature can be used as the reaction temperature, but the higher the temperature is, the more the addition reaction of the cationizing agent is promoted. Also, as concerns the pH of the system during the addition reaction, after the termination of the reduction reaction, there is no particular need to make pH adjustments. In other words, the reaction is conducted at a pH in the range of from 2 to 12, typically, in the range of from 6 to 11. As the addition reaction of the quaternary ammonium salt type cationizing agent with the reduction-treated keratinous substance proceeds, the keratinous substance dissolves in the reaction medium, with the final undissolved portion of it constituting 30% or less of the keratinous substance used as the raw material. The undissolved portion is removed by filtration, centrifugal separation, or other means, yielding a solution of modified keratin protein.

Upon removal of the reducing agent and other low molecular impurities from the thus obtained solution of modified keratin protein by means of ultrafiltration or dialysis, the solution can be used as is, but recovering the modified keratin protein in solid form by means of lyophilization is more convenient in terms of use as well as in terms of storage, transportation, etc.

Below, the present invention is illustrated specifically by referring to application examples.

Application Example 1

10g of wool fiber was immersed in 600g of an aqueous solution obtained by adding 0.02M Tris buffer and 6 ml of 2-mercaptoethanol was added thereto as a reducing agent, whereupon the pH was adjusted to 8.5 using 1N hydrochloric acid and a reduction reaction was conducted for 24 hours at room temperature in a stream of nitrogen. Next, 2.0g of glycidyltrimethylammonium chloride was added to the reaction system and the mixture was subjected to agitation for 6 hours at 50°C, solubilizing approximately 80% of the wool fiber in the reaction solution. The undissolved portion was removed by filtration while the reducing agent and other low molecular impurities were removed from the resulting aqueous solution of modified keratin protein by means of ultrafiltration (using a membrane with a molecular weight cut-off of 1,000), with the aqueous solution of modified keratin protein concentrated approximately 5 times in the process. 7.5g of modified keratin protein was obtained by lyophilizing it. The average molecular weight of the protein, obtained by gel filtration (using Sephadex G-75), was 41,000. In the following examples, the molecular weights were obtained in the same manner.

Application Example 2

7.8g of modified keratin protein with an average molecular weight of 40,000 was obtained by using the same procedure as in Application Example 1, with the exception of using a 50% aqueous solution of *n*-propanol as a reaction medium and 4 ml of tri-*n*-butylphosphine as a reducing agent.

Application Example 3

10g of wool fiber was immersed in 700g of a 30% aqueous solution of ethanol obtained by adding 0.02M Tris buffer and 4 ml of tri-*n*-butylphosphine was added thereto as a reducing agent, whereupon the pH was adjusted to 8.0 using 1N hydrochloric acid and a reduction reaction was conducted for 24 hours at room temperature in a stream of nitrogen. Next, 2.5g of allyltrimethylammonium chloride was added to the reaction system and the mixture was subjected to

agitation for 5 hours at 70°C, solubilizing approximately 85% of the wool fiber in the reaction solution. The undissolved portion was removed by filtration while the reducing agent and other low molecular impurities were removed by subjecting the resulting filtrate to ultrafiltration (using a membrane with a molecular weight cut-off of 1,000), with the filtrate concentrated to about 150 ml in the process. 8.2g of modified keratin protein with an average molecular weight of 39,000 was obtained by lyophilizing it.

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B6

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(54) A method for preparing a keratin substance with a low molecular weight

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Specification

1. Title of the Invention

A method for preparing a keratin substance with a low molecular weight

2. Scope of Patent Claims

1. A method for preparing a keratin substance with a low molecular weight, wherein when the keratin substance is reduced and then hydrolyzed, a reducing reaction is applied to said keratins while the state of the raw materials in the reaction medium is maintained before and after the reducing reaction, and then, a hydrolysis reaction is applied in the water using a protein hydrolase.

3. Detailed Description of the Invention

The present invention relates to a method for preparing soluble keratins with a low molecular weight (this is called "oligokeratin" in the present specification), wherein structural protein that comprises wool, down, hair, etc., is used as a raw material and the range of the molecular weight is from 200 to 10,000. To date, it is well known that low molecular weight protein (oligopeptides) obtained by partial hydrolysis of fibrous protein such as collagen, keratins, etc., is useful as a cosmetic base for the hair, etc. (see I. Bonadeo, et al., Cosmetics and Toiletries, 92, 45 (1977)). Partial hydrolysis by acid or alkali, degradation by protein hydrolase, and a method wherein reflux extraction is done at high temperature (thermal decomposition) with a liquid medium have been employed as methods for preparing these oligopeptides. Since it is particularly easy to control the molecular weight of the oligopeptides obtained with the method that employs hydrolase, its usefulness is widely known in the field of protein chemistry.

However, in the event that a keratin substance is used as a raw material, the enzymatic degradation the is rooted in the chemical structure thereof and the high level tissue structure is difficult, so the enzyme processing method that is well known in keratin chemistry results in a high cost for the product since an extremely complex operation is required for the separation and recovery of oligokeratins, and it is not something that is satisfactory in practical use. That is, keratins are insoluble since they are equipped with a natural cross-linked structure (disulfide bonds, ion cross-linking, hydrogen bonds, hydrophobic interactions, etc.), and moreover it is characteristically difficult to hydrolyze them with enzymes. In the case of wool, the proportion in which untreated wool is degraded by pronase is 20% or less, the proportion degraded by tripsin is 10% at the highest, and the proportion degraded by pepsin is about 5% (see P.H. Springel, Aust. J. Biol. Sciy., 16, 272 (1965), W.G. Crowther, et al., Advan. Protein Chem., 20, 191 (1965), W.G. Crowther, et al., J. Biol. Chem., 242 (1967)). Therefore, to date a method is known wherein the disulfide bond that makes the keratins insoluble is cleaved by the action of a reducing agent in the presence of a dissolution aid such as urea, and causing the enzyme to act once the keratins have been dissolved in the liquid medium, as a method for adjusting oligokeratins with hydrolase. However, according to this kind of method, a great deal of effort is required for elimination dissolution aids such as urea and the reducing agents by separation by filtration, ultrafiltration, dialysis, etc., of the undissolved part at the time of reduction by filtration.

As a result of earnest examination aimed at solving these problems the present inventors arrived at the present invention by discovering the fact that it is possible to obtain oligokeratins that are readily soluble in water and organic polar solvents in a manner that is extremely easy operationally and in high yields by just causing protein hydrolase to act on fibrous reduced keratins (keratine) that have been treated by reduction, without destroying the form of the raw materials, for example the shape of the raw wool.

That is, the present invention provides a method for preparing a keratin substance with a low molecular weight, wherein when the keratin substance is reduced and then hydrolyzed, a reducing reaction is applied to said keratins while the state of the raw materials in the reaction medium is maintained before and after the reducing reaction, and then, a hydrolysis reaction is applied in the water using a protein hydrolase.

The purpose of the present invention is to provide a new method for preparing oligokeratin that possesses superior performance as a cosmetic base, but the advantages and features of the present invention will probably become clear from the following explanation.

The characteristics of the present invention are that (1) a natural substance comprising keratins as the main ingredient are reduced and the disulfide bonds in said keratins are cleaved, but the reducing reaction is performed under conditions where the keratin substance is present in a state where the form of the raw materials in the reaction medium is maintained after the reaction as well, and (2) oligokeratins are prepared with a high yield by causing hydrolase alone to act directly, without employing a swelling agent or dissolution aid for keratin substances that have been used to date during enzyme hydrolysis, on the reduced keratin substance wherein the form of the above-mentioned raw materials is maintained.

The advantages of the present invention are that (1) the separation of reduced keratins from the reducing reaction medium is extremely easy, since the reduced keratin substance is present in a state wherein the form of the raw materials is maintained, even in the reducing reaction medium, and (2) the enzyme hydrolysis reaction can be conducted in an aqueous solution that contains only an extremely small amount of acid or alkali, which has been added for the purpose of making the pH of the hydrolase employed optimal, and as a result it becomes possible to omit such complex processes as ultrafiltration and dialysis in the separation and recovery of the oligokeratins that dissolve in the aqueous solution, and the separation and recovery of oligokeratins becomes extremely easy.

The oligokeratins provided by the present invention can be items that generally have a molecular weight in a range of 200 to 10,000, and a molecular weight in range of 500 to 5,000 under optimal conditions. In addition, the above-mentioned oligokeratins possess a thiol side change produced as a result of the fact that that at least one part of the disulfide bone that forms the cross-link of the polypeptide chain of the keratins is cleaved, and in addition to the functional groups such as the amino group, carboxyl group, and alcohol and phenol hydroxyl group that the polypeptide chain possesses originally it also possesses an amino group and a carboxyl group on the molecular chain terminal, and thus is extremely rich in functionality and has a superior performance as a cosmetic base.

A more concrete explanation of the method of the present invention is provided below.

Keratins are one kind of structural (tissue) protein that is present in large quantities in the tissue that develops in the skin of vertebrates and towards the exterior thereof; therefore, one can cite the horns, hooves, claws, body hair, down, etc., of vertebrates, and preferably wool and down, as the keratin substances used as the raw materials in the method of the present invention, and it is possible to use these as is or by pulverizing or cutting them to an appropriate size in accordance with need.

In addition, it is also possible to carry out pretreatment, such as washing, degreasing, etc., of these keratin substances as need dictates.

According to the present invention, the above-mentioned keratin substances are first reduced. The reduction can generally be performed in a liquid medium. The liquid mediums that can be used must be stable towards reduction, and the selection of the reduction system medium is extremely important so that the keratin substance retains the form of the raw material thereof even after the reducing reaction. That is, it is necessary to select for the liquid medium that is used in the present invention something that does not dissolve or suspend keratins before and after the reducing reaction. Water that does not contain a dissolution aid, alcohols such as methanol, ethanol, n-propanol and isopropanol, or a mixture of water and an alcohol, are optimal as such mediums.

During reduction, the keratin substance of the above-mentioned raw materials is immersed in the above-mentioned liquid medium. At that time, there is no stringent restriction on the weight ratio of the keratin substance to the liquid medium, but it is best that to make this ratio at least 1: 20, and ordinarily a range of 1: 30 to 1: 100, in accordance with the kind, shape, etc., of the keratin substance that is used.

Any reducing agent that one wishes can be used as the reducing agent provided that it can cause the disulfide bond in the keratin substance (– 3 – 8 –) **[?; partly illegible]** to cleave to the thiol group (– 8H) **[?; partly illegible].**

An organic and inorganic reducing agent of a type that acts neutrophilically towards the disulfide bond (– 3 – 8 –) **[?; partly illegible]** in general is preferable as a reducing agent that can be used. As the organic reducing agents, thiol derivatives and phosphorous compounds are suitable, and concretely such items as for example mercapto ethanol, mercaptoacetic acid, tributyl phosphine, triphenyl phosphine, etc., are used advantageously. In addition, such items as for example sodium hydrogensulfide ($NaHSO_3$) and sodium hydrosulfide (-NaSH) are optimal as the inorganic reducing agents.

The reduction itself can be carried out by the well-known methods (see W.G. Crowther, et al., Advan. Protein Chem., 20, 191 (1965), J.A. Maclaren, Aust. J. Chem., 15, 824 (1962), J.A. Maclaren, et al, Aust. J. Chem., 19, 2355 (1966)). For example, in the event that in the event that the above-mentioned thiol derivatives or inorganic reducing agents are used, it is advantageous that these are generally used in an excess equivalent, ordinarily in an equivalent of at least twice and preferably an equivalent of 4 to 10 times per one disulfide bond, relative to the disulfide bonds in the keratin substance used, and in addition in the event that the above-mentioned phosphorous compounds are used it is sufficient in general if these are used in an equivalent to a slightly excess equivalent relative to the disulfide bonds in the keratin substance used.

The amount of disulfide bonds in the keratin substance can be determined by amino acid analysis, and the method thereof is well known (M. Friedman, A.T. Noma, Textile Res. J., 40, 1073 (1970)).

The reduction can be performed under pH conditions of acidity, neutrality or alkalinity. However, swelling or dissolution of the keratin substances occur under somewhat high or low pH conditions, and it becomes impossible to retain the form of the raw materials of said reduced keratin substances, so ordinarily it is advantageous ordinarily to carry out the reduction in a pH range of 1 to 8.

There are no particular restrictions on the temperature and pressure during reduction, and it is possible to vary these over a wide range in accordance with the type of reducing agent and the type of raw materials used, but room temperature is adequate as the reducing temperature. The pressure during reduction and normal pressure are adequate, but it is also possible to carry out the reaction under reduced pressure or applied pressure in accordance with need.

In addition, the reduction can preferably be carried out in an inert gas atmosphere, for example in nitrogen.

By reduction like that described above, the disulfide bonds in the keratin substances are cleaved, but said keratin substances are present in a state wherein the form of the raw materials is maintained in the reducing medium. Therefore, it is possible to separate and recover the reduced keratin substances easily by such means as filtering and centrifugation, and to add the following enzyme treatment processes after the substances have been fully washed. During said separation operation and washing operation, it is necessary to pay attention so that the thiol side chains that are produced by the cleaving in the keratin substances are not oxidized in the separation operation, for example it is performed in an inert gas atmosphere, or it is preferable that the separation and cleaning operations are carried out in a steady state relative to the oxidation of said thiol side chains by adjusting the pH of said reducing medium or washing liquid to 6 or below.

The adjustment of the pH can be easily carried out by adding a water-soluble acidic substance, for example, inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, etc., or organic acids such as acetic acid, propionic acid, p-toluene sulfonic acid, etc. In particular, for reasons of ease of separation of the acid from the oligokeratins produced, a volatile acid, in particular hydrochloric acid, is preferable.

Enzyme hydrolysis treatment is then applied for the reduced keratin substances wherein the form of the raw materials obtained as described above is maintained. In the present invention, said enzyme hydrolysis is performed in an aqueous solution that is adjusted so that the inactivity of the hydrolase used becomes optimal, but it is one of the features of the present invention that said aqueous solution contains only a small amount of acid or alkali that is added in order to adjust the pH of the aqueous solution, and the hydrolase that is used, and this is also an advantage. It is possible to use an acidic enzyme such as pepsin and a neutral enzyme such as papain, pronase, tripsin, etc., as the above-mentioned hydrolase. However, the thiol side chains with reduced keratin are unstable when the pH of the aqueous medium is 6 or above and easily oxidized, and disulfide bonds are formed easily (see Shigeru **[illegible]**, "Chemistry of Organic Sulfur Compounds (Part 1)", *Kagaku Dojin*, Chapter 2, 1968). Therefore, it is desirable that hydrolysis be carried out with an acidic enzyme, preferably something like pepsin. In addition, it is necessary to restrict the temperature of the enzyme hydrolysis so that the enzyme that is used exhibits optimal activity. For example, a temperature range of 35 to 40 °C is preferable. The regulation of the molecular weight of the oligokeratins used can be carried out by selecting as appropriate the type, amount, reaction time and temperature of the enzyme that is used. In the event that deactivization of the enzyme used is necessary, this can be carried out by the methods that are well known in the field of enzyme chemistry, such as thermal treatment or pH adjustment.

In the event that insoluble foreign matter that adheres to the keratin and insoluble solid content is present, it is possible to carry out the separation and recovery of

oligopeptides from the oligopeptide aqueous solution that is obtained as described above by removing these by such means as filtration and centrifugation, after which an ordinary method is carried out such as for example freeze drying after said keratin aqueous solution is concentrated.

The oligokeratins obtains in this manner are white to light yellow powders, and it is possible to employ these for uses as a cosmetic base, and in particular a cosmetic base for hair.

As described above, when preparing oligopeptides, to date a refining process such as ultrafiltration or dialysis was indispensable, and thus a large plant for preparing oligopeptides was required on an industrial scale since said refining process was necessary, and a great deal of time was required as well, but the present invention does not require said refining process, nor does it require a large plant or a long time, and thus it possible for the first time to prepare oligopeptides with an extremely simple process.

A more concrete explanation is provided below of the present invention by citing working examples.

Working Example 1

10 g wool fiber was immersed in 1,000 cc of a 20% (W/W) n-propyl alcohol aqueous solution, and an adequate amount of nitrogen gas was caused to flow into the solution. Then, 2.5 ml tri-n-butyl phosphine was added, and then it was shaken lightly for 1 day at room temperature. At this point in time, the wool retained its original fibrous state, and after it was filtered and washed with ethanol, it was subjected to drying under reduced pressure at 30 °C, and fibrous reduced wool was obtained. 10 g fibrous reduced wool that was obtained in this manner was immersed in 1,000 cc 0.0075 N hydrochloric acid (pH 1.3), 200 mg pepsin was added and then it was shaken for 1 day at 40 °C, and the hydrolysis reaction was carried out. As the hydrolysis reaction progresses, the wool fibers dissolved in the hydrochloric acid, and 3 to 4 hours later there was almost no undissolved portion left. After the oligokeratin hydrochloric acid solution obtained in this manner was filtered, it was concentrated to approximately 300 cc with an evaporator, and 9.8 g of a light yellow powder oligokeratin was obtained by freeze drying (solubility rate 98%). Said oligopeptide was carboxymethylized with iodoacetic acid, and when its molecular weight was calculated by gel filtration (using G-50 Sephadex) it was 1,100. The molecular weights were calculated in the same manner in the following working examples.

Reference Example 1

10 g of untreated wool fiber to which reduction was not applied was immersed in 1,000 cc 0.075 N hydrochloric acid, and it was shaken for 1 day at 40 °C and a hydrolysis reaction was carried out. At this point in time, a majority of the fibrous wool remained, and when this was filtered, washed with ethanol and dried it was 9.2 g. In other words, the solubility rate was 8%.

Working Example 2

The same operation as in Working Example 1 was carried out, except for the fact that 15 ml mercaptoacetic acid was used as the reducing agent instead of the tri-n-butyl phosphine, and 1,000 cc water was used as the reducing reaction dispersion agent instead of the 20% n-propyl alcohol aqueous solution, in Working Example 1. The result thereof was that a light yellow powder oligokeratin with a solubility rate of 95% was obtained. Its molecular weight was 1,000.

Working Example 3

The same operation as in Working Example 1 was carried out, except for the fact that 15 ml 2-mercapto ethanol was used as the reducing agent instead of the tri-n-butyl phosphine, and 1,000 cc water was used as the reducing reaction dispersion medium instead of the 20% n-propyl alcohol aqueous solution, in Working Example 1. The result thereof was that a light yellow powder oligokeratin with a solubility rate of 90% was obtained. Its molecular weight was 1,000.

Working Example 4

The same operation as in Working Example 1 was carried out, except for the fact that 20 g sodium hydrogensulfide was used as the reducing agent instead of the tri-n-butyl phosphine, and 1,000 cc water was used as the reducing reaction dispersion medium instead of the 20% n-propyl alcohol aqueous solution, in Working Example 1. The result thereof was that a light yellow powder oligokeratin with a solubility rate of 92% was obtained. Its molecular weight was 1,100.

Working Example 5

The same operation as in Working Example 1 was carried out, except for the fact that the pH of the hydrolysis reaction medium was set at 6.8 by employing 200 mg papain as the hydrolase instead of pepsin in Working Example 1. The result thereof was that a light yellow powder oligokeratin with a solubility rate of 50% was obtained. Its molecular weight was 1,500.

Working Example 6

The same operation as in Working Example 1 was carried out, except for the fact that the pH of the hydrolysis reaction medium was set at 7.2 by employing 200 mg pronase P as the hydrolase instead of pepsin in Working Example 1. The result thereof was that a light yellow powder oligokeratin with a solubility rate of 85% was obtained. Its molecular weight was 1,500.

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